Factors Affecting Storage Stability of Various Commercial Phytase Sources[1]

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Summary

A 360-d study was performed to evaluate the effects of environmental conditions on storage stability of exogenous phytases. Coated and uncoated products from 3 phytase sources (Ronozyme P, OptiPhos, and Phyzyme) were stored as pure forms, in a vitamin premix, or in a vitamin and trace mineral (VTM) premix. Pure products were stored at 0, 41, 73, and 99°F (75% humidity). Premixes were stored at 73 and 99°F. Sampling was performed on d 0, 30, 60, 90, 120, 180, 270, and 360. Sampling of the pure products stored at 0 and 41°F was discontinued after d 120 due to mold growth in the 41°F samples. Stability was measured as the residual phytase activity (% of initial) at each sampling point. For the stability of the pure forms, all interactive and main effects of phytase product, coating, time, and temperature of storage were significant ($P < 0.01$), except for time × coating interaction. When stored at 73°F or less, pure phytases retained at least 91, 85, 78, and 71% of initial phytase activity at 30, 60, 90, and 120 d of storage, respectively. However, storing pure products at 99°F reduced ($P < 0.01$) phytase stability, with OptiPhos retaining the most ($P < 0.01$) activity. Coating mitigated ($P < 0.01$) the negative effects of high storage temperature for Ronozyme and OptiPhos (from d 90 onward) but not for Phyzyme. For the stability of phytase in different forms of storage, all interactive and main effects of phytase product, form, coating, time, and temperature of storage were significant ($P < 0.01$). When stored at room temperature (73°F), retained phytase activities for a majority of the phytase sources were more than 85, 73, and 60% of initial activity up to 180 d when stored as pure products, vitamin premixes, or VTM premixes, respectively. When stored at 99°F, pure phytase products had greater ($P < 0.01$) retention of initial phytase activity than when phytases were mixed with the vitamin or VTM premixes. Coated phytases stored in any form had greater ($P < 0.01$) activity retention than the uncoated phytases at all sampling periods. In conclusion, storage stability of commercially available phytases is affected by duration of storage, temperature, product form, coating, and phytase source. Pure products held at 73°F or less were the most stable. In premixes, longer storage time and higher temperature reduced phytase activity, but coating mitigated some of these negative effects.

Key words: enzyme, phytase, stability, storage

[1] Appreciation is expressed to DSM Nutritional Products, Inc., Parsipanny, NJ, and Enzyvia, LLC, Sheridan, IN, for providing the phytase analyses.
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Introduction

Phytases are routinely used in swine and poultry diets as an economical P source to increase the availability of phytate phosphorus in the diet. The rapid increase in phytase use has led to the introduction of several commercial phytases produced from various microbial sources. The ultimate value of any phytase product depends on its efficacy and stability. As with any catalytic proteins, phytases lose significant amount of activity when subjected to feed processing treatments. Research has focused on optimizing thermostability of exogenous phytases in industrial settings. Nutritionists most often consider minimum guaranteed levels of phytase after feed processing in their diet formulations; however, the stability of phytases during storage receives little attention. Currently, there has been no independent study evaluating the effects of various factors such as coating, time, or temperature of storage on the stability of commercial phytases. In addition, the use of phytase-fortified vitamin and vitamin-trace mineral premixes is becoming more popular in the industry. There also may be potential interactions between phytase and some components of the premixes that may affect phytase activity.

Therefore, the objective of this study was to determine the effects of coating, storage form, storage temperature, and duration of storage on the stability of six commercially available phytases.

Procedures

This study was conducted at the Animal Nutrition Laboratory and at the Bioprocessing and Industrial Value Added Program (BIVAP) Building at Kansas State University.

Phytase sources

Six commercially available phytases were used in this experiment: OptiPhos 2000-M (uncoated, declared potency of 2,000,000 phytase units [FTU]/kg); OptiPhos 2000-PF (coated, declared potency of 2,000,000 FTU/kg); Phyzyme XP 5000 G (uncoated, declared potency of 5,000,000 FTU/kg); Phyzyme XP 10,000 TPT (coated, declared potency of 10,000,000 FTU/kg); Ronozyme P-M (uncoated, declared potency of 50,000,000 phytase units [FYT]/kg); and Ronozyme P-CT (coated, declared potency of 10,000,000 FYT/kg). One phytase unit (FTU or FYT) was defined as the amount of enzyme that catalyzes the release 1 μmol of iP per minute from 5.1 mM sodium phytate in pH 5.5 buffer at 37°C. Pure cornstarch was used as a negative control due to the low inherent phytase activity. The coated and uncoated phytases were obtained from a third-party distributor. The manufacturing dates of all products were obtained from the original supplier to ensure that the products were within 6 mo of manufacture and were not expired.

Pure products

On d 0, 3 lb of each of the pure phytase products and cornstarch were individually placed into 12 open, single-lined paper bags. Three bags of each product were stored in a freezer (0°F), in a refrigerator (41°F), at room temperature (73°F), and in a controlled environment chamber set at 99°F and 75% humidity. A blind sample from each bag was taken at d 30, 60, 90, 120, 180, 270, and 360, and sent to Technical Marketing Analytical Services of DSM Nutritional Products, Inc. (Belvidere, NJ) for phytase analysis using a slight modification of the AOAC official method (AOAC, 2000). A second sample from each bag of the cornstarch control, OptiPhos 2000-M, and Opti-

Phos 2000-PF was blinded and sent to Phytex, LLC (Portland, ME) for phytase analysis using the Phytex method. However, sampling of the pure products stored at 0 and 41°F was discontinued after d 120 due to mold growth in the retained 41°F samples. Thus, only pure products stored at 73 and 99°F were sampled for all time points.

Premixes

Each phytase product and the cornstarch control were added and mixed with either the K-State vitamin premix or the vitamin and trace mineral premix (VTM). The amount added for each phytase product was determined such that including 0.30% premix in the diet would provide the levels of phytase recommended by its respective manufacturer (250 FTU/kg, OptiPhos 2000-M and OptiPhos 2000-PF; 500 FTU/kg, Phyzyme XP 5000 G and Phyzyme XP 10,000 TPT; 1,850 FYT/kg, Ronozyme P-M and Ronozyme P-CT).

A total of 5.4, 5.4, 3.0, 1.5, 5.6 and 1.1 lb of pure OptiPhos 2000-M, OptiPhos 2000-PF, Phyzyme XP 5000-G, Phyzyme XP 10000 TPT, Ronozyme P-M, and Ronozyme P-CT, respectively, were weighed. Cornstarch was added to the pure phytase products to create 21.7-(OptiPhos) or 15.0-(Phyzyme XP and Ronozyme P) lb batches, which were mixed with a paddle mixer for 5 min. A total of 108 (OptiPhos) or 75 (Phyzyme XP and Ronozyme P) lb of vitamin or VTM premix was added to each batch and mixed with a paddle mixer for an additional 12 min to create premix batches of 130 (OptiPhos) or 90 (Phyzyme XP and Ronozyme P) lb. Additionally, 130 lb of cornstarch made up the control batch. The vitamin premix was that recommended by K-State. The VTM contained equal quantities of K-State-recommended vitamin and trace mineral premixes.

The 7 batches were each equally divided into 6 open, single-lined paper bags. Three bags of each batch were stored either at room temperature (approximately 73°F) or in the environmentally-controlled chamber set at 99°F and 75% humidity. A sample from each bag was taken every 30 d until d 180, except for the last 2 samplings (taken at d 270 and 360). Each blind sample was sent for phytase analysis to Technical Marketing Analytical Services of DSM Nutritional Products, Inc., using a slight modification of the AOAC official method. A second sample from each bag containing the control, OptiPhos 2000-M, and OptiPhos 2000-PF premixes, was blinded and sent to Phytex, LLC for phytase analysis using the Phytex method.

Statistical analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) to determine the interactive and main effects of coating, storage form, storage temperature, and time on stability of six commercially available phytases. Because the vitamin and VTM premixes were only stored at room temperature and in the environmentally controlled heat chamber, 2 analyses were performed. The first was with the pure forms only, and the second was for pure forms, vitamin, and VTM premixes at 73°F and 99°F. Least square means were calculated for each independent variable. When treatment effect was a significant source of variation, differences were determined by using the preplanned, pairwise comparisons (PDIFF option of SAS). Statistical significance and tendencies were set at $P \leq 0.05$ and $P < 0.10$ for all statistical tests.

Results

Initial phytase activity

The calculated and analyzed initial (d 0) phytase activity of the samples is shown in Table 1. Using the AOAC assay, the control samples for the pure product, the vitamin premix, and the VTM premix contained 4,967 to 10,500 phytase units/kg. However, the phytase activity of the control samples analyzed using the Phytex assay was much higher than the analyses using the AOAC assay. For all three forms, the AOAC-analyzed phytase levels of OptiPhos, Phyzyme XP, and Ronozyme P were 196 to 295, 97 to 157, and 103 to 142% higher than their calculated phytase levels. Using the Phytex assay, samples of the pure OptiPhos 2000-M and OptiPhos 2000-PF had similar (101 to 102%) phytase activity compared to their calculated levels. In contrast, phytase activity of both OptiPhos products added to the vitamin and the VTM premix were lower, ranging from 30 to 68% of their calculated levels.

Pure products

All interactive and main effects of phytase product, coating, time, and temperature of storage were significant ($P < 0.01$; Table 2), except for time × coating interaction.

When stored at 73°F or less, the retained activity of phytases stored in pure form decreased ($P < 0.01$) as storage duration increased, regardless of phytase source or coating (Figures 1 to 3). At d 30, 60, and 90, pure phytases retained at least 91, 85, and 78% of initial phytase activity, respectively. Until d 120, the pure forms retained 71 to 102% of initial phytase activity, except for Ronozyme M, which retained 59% at 41°F. However, storing pure products at 99°F had greater ($P < 0.01$) effects on phytase stability. At d 30, both OptiPhos products stored in pure form retained 91 to 93% of initial activity when stored at 99°F, whereas the Phyzyme phytases retained 69 to 74%. Ronozyme CT retained 69% of initial phytase activity at d 30, but Ronozyme M only retained 36%. Afterward, phytases stored in pure forms retained at least 44, 39, and 33% of initial phytase activity at d 60, 90, and 120, respectively, except for Ronozyme M. Ronozyme M retained only 5% of initial phytase activity at d 120. At d 180, 270, and 360, phytases stored in pure forms at 99°F had retained phytase activities ranging from 1 to 53%, compared with 50 to 109% when stored at 73°F.

The coated OptiPhos had similar retention rates compared with the uncoated OptiPhos at d 30 and 60 when stored at 99°F, but coating improved ($P < 0.01$) its retention rates from d 90 onward. Coating also improved ($P < 0.01$) the retained phytase activities of Ronozyme phytase throughout the study; however, the coated Phyzyme had lower ($P < 0.01$) phytase activities than the uncoated Phyzyme until d 360. Among the coated phytases, the retention rates of Ronozyme-CT were lower ($P < 0.01$) than OptiPhos 2000-PF until d 120, while it was similar with Phyzyme 10,000 TPT until d 90. Among the uncoated phytases, OptiPhos 2000-M had greater ($P < 0.01$) phytase activities than both Phyzyme 5,000 G and Ronozyme M at d 30, but Phyzyme 5,000 G retained more ($P < 0.01$) than the other 2 uncoated phytases from d 90 onward.

Premixes

All interactive and main effects of phytase product, form, coating, time, and temperature of storage were significant ($P < 0.01$; Table 3), except for time × form × coating and coating × temp interactions ($P < 0.08$).

When stored at 73°F, pure forms retained more ($P < 0.01$) phytase activity with increasing duration of storage than phytase-supplemented vitamin or VTM premixes (Figures 4 to 6). Pure phytase products retained at least 85 and 72% of initial phytase activity until d 180 and d 360, respectively; except for Ronozyme M (50%). In contrast, phytase-supplemented vitamin premixes retained at least 73% until d 180, except for Phyzyme 5,000 G (67%). At d 270 and d 360, both Phyzyme 5,000 G and Ronozyme M retained 56 to 59% of initial phytase activity, while the rest of the phytases retained at least 68%. Among all the phytases, OptiPhos 2000 PF retained the most activity (> 92%; $P < 0.01$) until d 360 when mixed with the vitamin premixes. In comparison, Ronozyme CT retained at least 83% of its initial phytase activity, whereas Phyzyme 10,000 TPT retained at least 73% until d 360. For the phytase-supplemented VTM premixes, retained phytase activities were at least 60% until d 180, except for OptiPhos 2000 M (43%). At d 270 and d 360, OptiPhos 2000 M only had 28% of its initial phytase activity, compared with at least 52% for the rest of phytases when mixed into the VTM premixes. As with the vitamin premixes, OptiPhos 2000 PF retained the most activity ($P < 0.01$) among all the phytases when mixed into the VTM premixes; however, its retention rates were lower ($P < 0.01$) than the rates obtained in the vitamin premixes. At d 360, OptiPhos 2000 PF, Ronozyme CT, and Phyzyme 10,000 TPT retained at least 83, 75, and 63% of initial phytase activity, respectively.

When stored at 99°F, retained phytase activities were much lower ($P < 0.01$) than the retention rates observed in samples stored at 73°F, regardless of the phytase source, coating, or form of storage. Pure phytase products also had greater ($P < 0.01$) retained phytase activities than the phytase-supplemented vitamin or VTM premixes. For the phytase-supplemented vitamin and VTM premixes, retained phytase activities after only 30 d of storage was 59 and 62% on average, which is lower ($P < 0.01$) than 72% for the pure phytase products. Ronozyme M was the least stable when mixed into vitamin premixes, retaining only 31% of its initial phytase activity at d 30. For the VTM premixes, OptiPhos 2000 M was the most affected, retaining only 20% of its initial phytase activity after a month of storage. At d 180, the phytase treatments had 3 to 53% of initial phytase activity. At the end of study (d 360), all the phytases had less than 28% of initial phytase activity.

The coated phytases stored in pure form or phytase-supplemented vitamin or VTM premixes had greater ($P < 0.01$) phytase activity than the uncoated phytases at all sampling periods. However, the differences in phytase activity between the coated and uncoated phytases were smaller ($P < 0.01$) when they were stored in pure forms than in the vitamin and VTM premixes. At d 30, 60, and 90, the differences in retained phytase activity between the coated and uncoated phytases ranged from 4.2 to 4.5, 11.5 to 28.6, and 33.4 to 44 percentage units when the phytases were in pure forms, vitamin premixes, and the VTM premixes. At d 30, coated phytases had similar phytase activities between the 3 forms when stored at 99°F; however, uncoated phytases stored in pure form had greater ($P < 0.01$) phytase activity than those mixed with the vitamin and VTM premixes. Likewise, uncoated phytases in vitamin premixes retained greater ($P < 0.01$) phytase activity than those in VTM premixes. When uncoated phytases were used and stored at 99°F, the pure forms had greater ($P < 0.01$) phytase activities than those in vitamin premixes, while both had greater ($P < 0.01$) phytase activities than the VTM premixes at all sampling periods.

Discussion

Phytase assays

Previous research at Kansas State University (Jones, et al. 2009[5]) demonstrated that the level of accuracy of the analysis for phytase activity depended on the phytase product and assay method used. Using the AOAC method, the initial phytase activity of OptiPhos was 2 to 3 times greater than levels calculated by the manufacturer, which is similar (2.5 times) to the difference observed in earlier research. The analyzed initial phytase activities for Phyzyme and Ronozyme were closer (1 to 1.6 times greater) to their calculated levels, which is expected, as the AOAC assay is the recommended method of analysis for these products. For Optiphos, the analyzed initial phytase activity was similar to the manufacturer's calculated levels when their recommended Phytex assay was used.

Phytases in pure forms

Phytase manufacturers often provide overages as much as 10 to 30% in phytase activity to account for potential losses during feed processing treatments and storage. However, data are limited on the storage stability (defined as % of initial phytase activity) of commercial phytases, except for those reported by manufacturers in product registrations (European Food Safety Authority, 2006[6]; 2008[7]; 2009[8]). Though temperatures and conditions from manufacture, transport, and storage of phytases may not approximate conditions during feed processing, enough variation exists in storage conditions and time among phytase users to expect further losses in phytase activity. Most nutritionists do not measure phytase activity at the time of use, thus, it is important to understand the stability of the different commercial phytases during storage as affected by temperature and time.

The results of this study demonstrated that when phytase is stored at room temperature (73°F) or less, the pure product retained most (~85%) of its activity up to 60 d of storage, regardless of the phytase source or coating. However, phytase source influenced stability when storing the product for more than 60 d at 73°F or less, with Optiphos and Phyzyme retaining more activity than Ronozyme. In the current study, Phyzyme XP 5000G and Phyzyme XP 10000 TPT retained 90.9 and 86.3% of initial activity, respectively, when stored at 73°F and 180 d, which is similar to the retention rates reported to the European Food Safety Authority (2006, 2008). In these reports, the product had 87 and 80% of initial activity after 365 d of storage at 68°F. However, the current results did not confirm the retention rates reported for Ronozyme M (European Food Safety Authority, 2009). After 180 d, it was reported that Ronozyme M retained 99 and 90% of initial phytase activity when stored at 50 and 77°F, respectively, which is greater than our observations (58.7% for 120 d at 41°F and 60.6% after 180 d at 73°F).

Storing phytase in ambient temperatures greater than 99°F and 75% relative humidity was detrimental to the stability of the pure product. More importantly, phytase source affected retention rates with increasing time of storage, with the highest rates recov-

[5] Jones et al., Swine Day 2009, Report of Progress 1020, pp. 106-121.
[6] European Food Safety Authority. 2006 The EFSA Journal 404:1-20.
[7] European Food Safety Authority. 2008. The EFSA Journal 915:1-10.
[8] European Food Safety Authority. 2009. The EFSA Journal 1097:1-20.

ered from OptiPhos, followed by Phyzyme, and finally, by Ronozyme. This ranking among the three phytase sources was the same throughout the study. The difference in retained phytase activities between OptiPhos and Ronozyme was large (91.5 vs 52.6% after 30 d, 45.8 vs 8.9% after 180 d). In the European Food Safety Authority report (2009), Ronozyme M kept at 104°F and 60% relative humidity retained only 50% of its initial phytase activity after 30 d, which is similar to the rate retained in the current study. The stability limit of *Escherichia coli* phytases, such as OptiPhos and Phyzyme, has been reported to be 140°F, whereas the stability limit for *Peniophora lycii* phytases has been reported at 176°F. Both temperatures are greater than the heat treatment used in this study; however, one major difference is that these thermal stability rates were determined by incubating the enzyme at low pH for a short duration of time (~30 min) whereas the enzyme was subjected to lower but sustained heat for a longer duration (up to 180 d) in this study. Another factor may be the high humidity (75%) in the chambers in our study. Others have evaluated the effects of increasing ambient humidity (from 53 to 90%) on the stability of commercial phytases stored at high ambient temperatures (104°F) for 70 d, and observed that phytase activity decreased significantly with increasing ambient humidity. This suggests that regardless of the phytase source, the environmental conditions set in the current study were sufficient to denature the enzyme and reduce activity. These conditions do not attempt to mimic real conditions during transport of the product or storage where temperatures and humidity may be more variable, but it clearly demonstrates the importance of maintaining better conditions (e.g., 73°F or less and lower ambient humidity) during storage to achieve greater stability from phytase products

Overall, coated pure products had greater phytase activity than uncoated pure products when exposed to 99°F and increasing storage time, but this differed between phytase sources. Coating was beneficial for Ronozyme and OptiPhos (only from d 90 onward) but not for Phyzyme, wherein the uncoated product retained more activity than the coated product throughout the study. This suggests that the type of coating may differ between phytase manufacturers, and that some coated phytase products may provide better protection during storage than others.

Phytases in premixes

For most of the commercial phytase sources tested, retained phytase activities were more than 85, 73, and 60% of initial activity up to 180 d when stored as pure products, vitamin premixes, or VTM premixes, respectively, and when storage temperatures were at 73°F. The exceptions were Ronozyme M for the pure phytase products, Phyzyme 5000 G for the vitamin premixes, and OptiPhos 2000 M for the VTM premixes, which are all uncoated phytases. In general, greater retention was observed with increasing storage time when phytases were stored as pure products than when mixed into either of the premixes. This suggests that storing phytase in pure forms may have advantages in retaining its original phytase activity compared with including it in premixes, when stored at room (73°F) or lower temperatures.

When phytase was mixed into vitamin or VTM premixes and exposed to heat treatment (99°F), coated phytases retained greater activities than uncoated phytases, especially when stored for more than 90 d. However, there were some differences between phytase sources, where coating had the greatest benefits for OptiPhos. Results also showed that uncoated phytases have very poor stability when mixed into the premixes

and stored for even as few as 30 d. The loss of phytase activity was greater when phytase was mixed with VTM premixes than with vitamin premixes. These results suggest that high heat and humidity, as well as potential interactions with some components of the premixes, increased the rate of denaturation of phytases. Previous work has shown that mixing inorganic trace minerals with vitamins leads to significant losses in vitamin activity, which is thought to be due to the presence of ionic charges in mineral salts that can act as oxidizing agents. It is not the objective of the study to identify specific vitamins or trace minerals that may have contributed to greater losses in phytase activity, but the results clearly indicate that coated phytases should be used in premixes. This also demonstrates the differences in the ability of coating technologies to protect phytases not only from environmental degradation, but also against the negative effects of certain components in vitamin and VTM premixes.

In conclusion, stability of commercially available phytases during storage is affected by numerous factors, such as storage time, temperature, product form, coating, and source. Pure phytase products stored at 73°F or less were the most stable. In premixes, longer storage time and higher temperature reduced phytase activity, but coating mitigated some of these negative effects.

Table 1. Calculated and analyzed phytase composition of samples at d 0[1]

	Phytase composition				
Item	Calculated, PU/kg[2]	AOAC analysis, PU/kg	AOAC ratio[3]	Phytex analysis, PU/kg	Phytex ratio[4]
Pure product					
Control[5]	0	10,500	---	3,343,000	---
OptiPhos 2000-M[6]	2,000,000	3,932,000	1.96	2,046,000	1.02
OptiPhos 2000-PF[6,9]	2,000,000	5,179,000	2.58	2,022,000	1.01
Phyzyme 5000 G[7]	5,000,000	5,144,000	1.03	---	---
Phyzyme 10000 TPT[7,9]	10,000,000	10,587,000	1.06	---	---
Ronozyme P-M[8]	50,000,000	52,148,500	1.04	---	---
Ronozyme P-CT[8,9]	10,000,000	12,057,500	1.20	---	---
Vitamin premix					
Control[5]	0	4,967	---	37,000	---
OptiPhos 2000-M[6]	83,333	214,425	2.51	41,000	0.49
OptiPhos 2000-PF[6,9]	83,333	250,853	2.95	57,000	0.68
Phyzyme 5000 G[7]	166,666	266,339	1.57	---	---
Phyzyme 10000 TPT[7,9]	166,666	266,116	1.57	---	---
Ronozyme P-M[8]	616,666	738,388	1.19	---	---
Ronozyme P-CT[8,9]	616,666	637,467	1.42	---	---
Vitamin and trace mineral premix					
Control[5]	0	4,948	---	77,000	---
OptiPhos 2000-M[6]	83,333	209,424	2.45	25,000	0.30
OptiPhos 2000-PF[6,9]	83,333	244,067	2.87	55,000	0.66
Phyzyme 5000 G[7]	166,666	209,437	1.23	---	---
Phyzyme 10000 TPT[7,9]	166,666	166,239	0.97	---	---
Ronozyme P-M[8]	616,666	699,542	1.13	---	---
Ronozyme P-CT[8,9]	616,666	877,884	1.03	---	---

[1] Values represent means of 3 replicates sampled in duplicate. AOAC analysis was performed at DSM Nutritional Products laboratory (Belvidere, NJ) while the Phytex analysis was performed at Phytex LLC (Sheridan, IN).
[2] PU = phytase units
[3] Ratio of average AOAC analyzed values to calculated values.
[4] Ratio of Phytex analyzed values to calculated values.
[5] Cornstarch used as the negative control.
[6] Phytex LLC, Sheridan, IN.
[7] Danisco Animal Nutrition, Marlborough, UK.
[8] DSM Nutritional Products, Basel, Switzerland.
[9] Coated phytase.

Table 2. Probabilities of interactive and main effects of storage time, temperature, coating, and phytase product on stability (as defined by % of initial phytase activity) of commercially available phytase sources in pure forms.

Item	*P*-value
Interactive effects	
Time × Temp × Coating × Product	<0.0001
Time × Temp × Product	<0.0001
Time × Temp × Coating	<0.0001
Time × Coating × Product	<0.0001
Temp × Coating × Product	<0.0001
Temp × Coating	<0.0001
Temp × Product	<0.0001
Time × Temp	<0.0001
Time × Coating	0.428
Time × Product	<0.0001
Coating × Product	<0.0001
Main effects	
Time	<0.0001
Temp	<0.0001
Coating	<0.0001
Product	<0.0001

Table 3. Probabilities of interactive and main effects of storage time, form, temperature, coating, and phytase product on stability (as defined by % of initial phytase activity) of commercially available phytase sources.

Item	*P*-value
Interactive effects	
Time × Form × Coating × Product × Temp	< 0.0001
Time × Form × Coating × Product	< 0.0001
Time × Form × Coating × Temp	< 0.0001
Time × Form × Product × Temp	< 0.0001
Time × Coating × Product × Temp	< 0.0001
Form × Coating × Product × Temp	< 0.0001
Time × Form × Coating	< 0.0721
Time × Form × Product	< 0.0001
Time × Form × Temp	< 0.0001
Time × Coating × Product	< 0.0001
Time × Coating × Temp	< 0.0001
Time × Product × Temp	< 0.0003
Form × Coating × Product	< 0.0001
Form × Coating × Temp	< 0.0004
Form × Product × Temp	< 0.0001
Coating × Product × Temp	< 0.0001
Time × Form	< 0.0001
Time × Coating	< 0.0028
Time × Product	< 0.0001
Time × Temp	< 0.0001
Form × Coating	< 0.0001
Form × Product	< 0.0001
Form × Temp	< 0.0001
Coating × Product	< 0.0001
Coating × Temp	< 0.0829
Product × Temp	< 0.0001
Main effects	
Time	< 0.0001
Form	< 0.0001
Coating	< 0.0001
Product	< 0.0001
Temp	< 0.0001

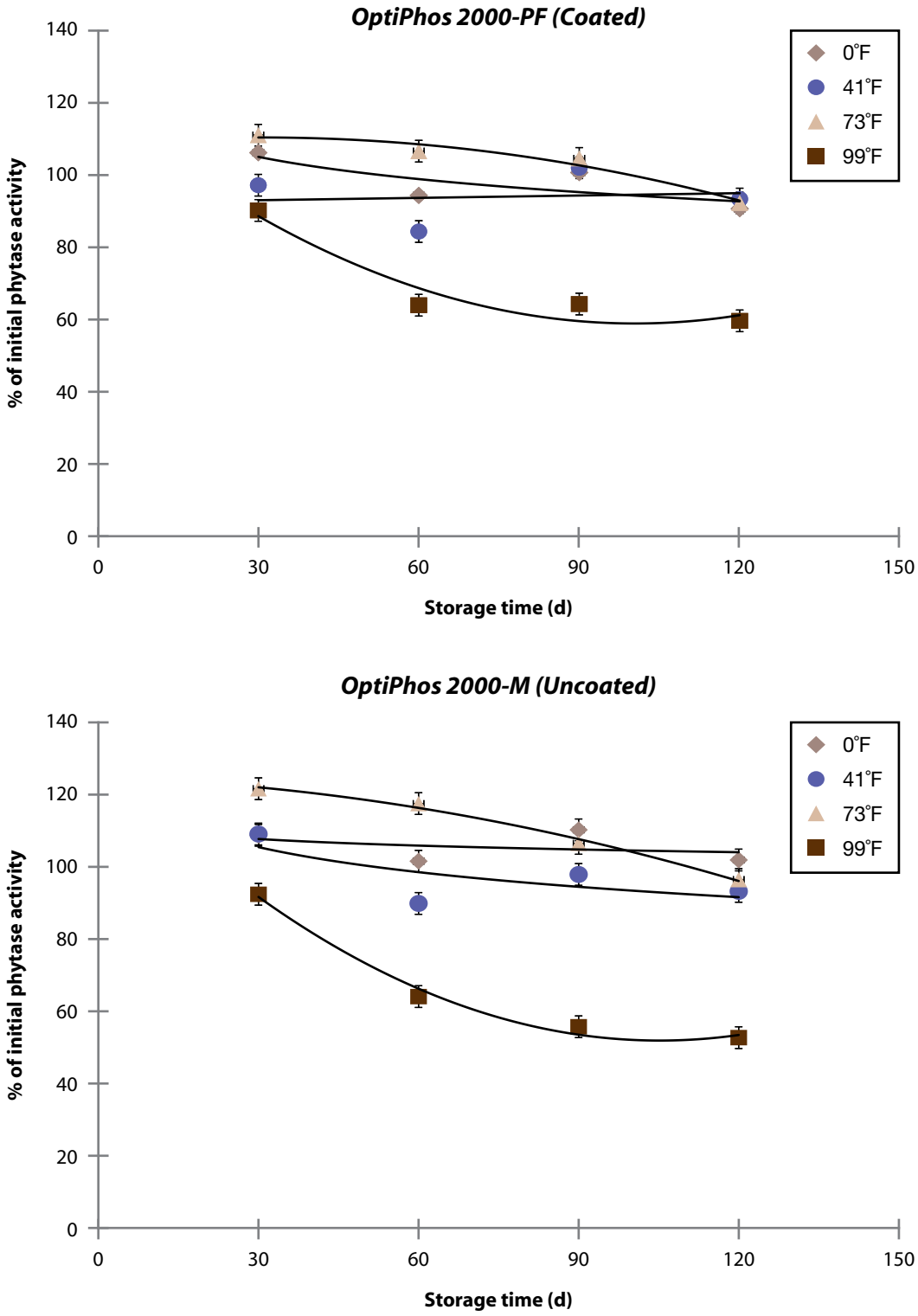

Figure 1. Residual phytase activity (% of initial) for OptiPhos 2000-PF (coated) and OptiPhos 2000-M (uncoated) as affected by storage temperature (freezer [0°F], refrigerator [41°F], at room temperature [73°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 120 d). Each data point (least square mean ± 2.32) is the mean of 3 observations.

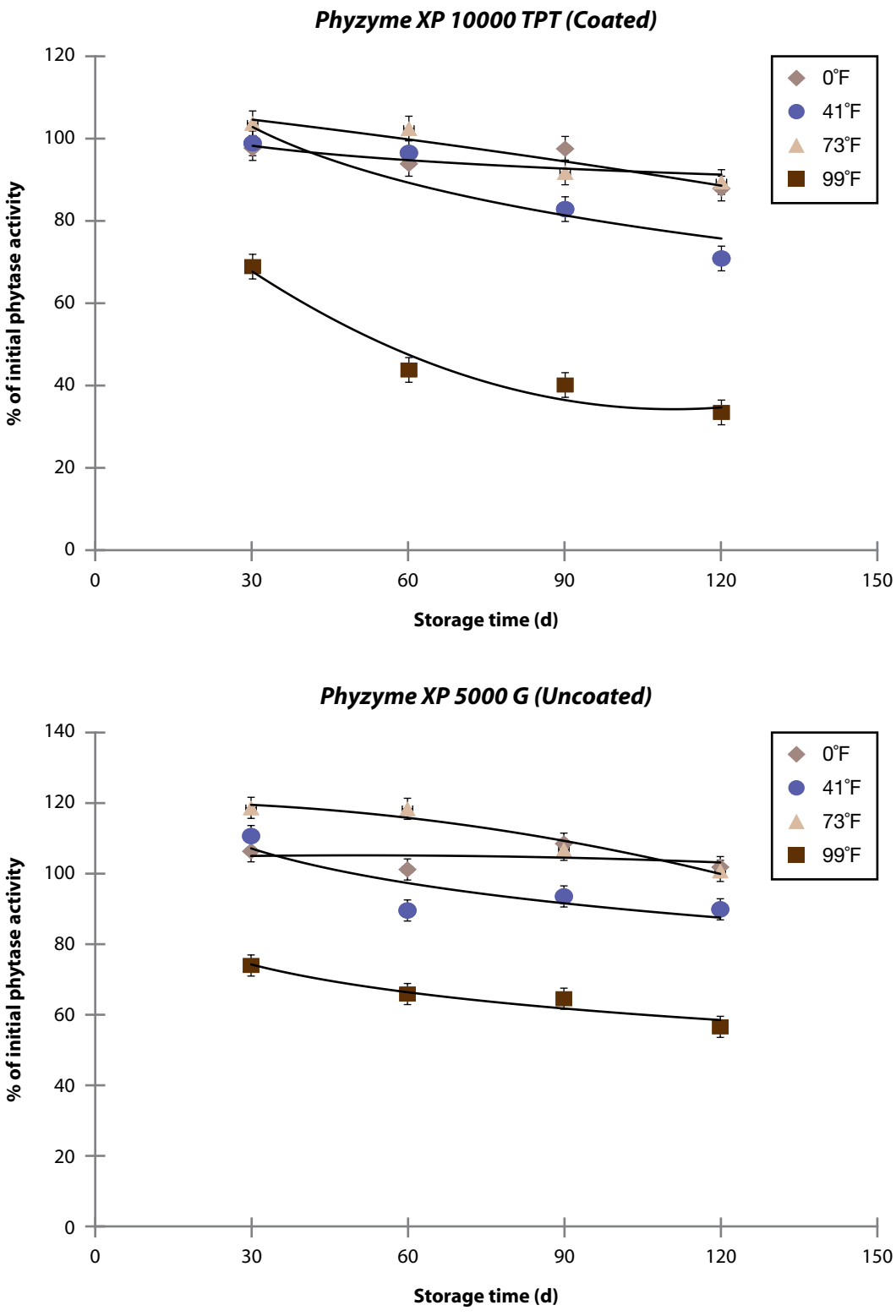

Figure 2. Residual phytase activity (% of initial) for Phyzyme 10000 TPT (coated) and Phyzyme 5000G (uncoated) as affected by storage temperature (freezer [0°F], refrigerator [41°F], at room temperature [73°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 120 d). Each data point (least square mean ± 2.32) is the mean of 3 observations.

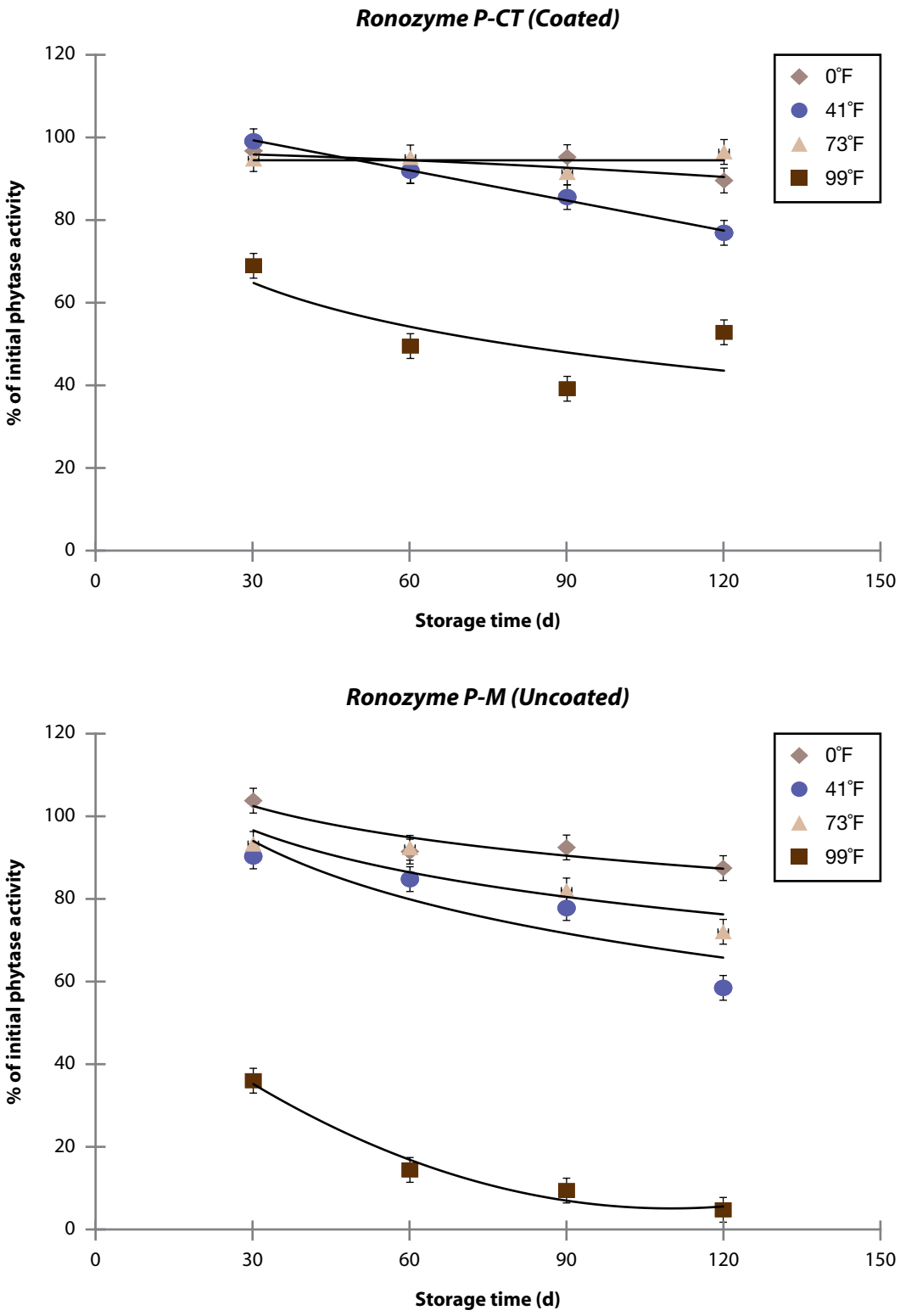

Figure 3. Residual phytase activity (% of initial) for Ronozyme CT (coated) and Ronozyme M (uncoated) as affected by storage temperature (freezer [0°F], refrigerator [41°F], room temperature [73°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 120 d). Each data point (least square mean ± 2.32) is the mean of 3 observations.

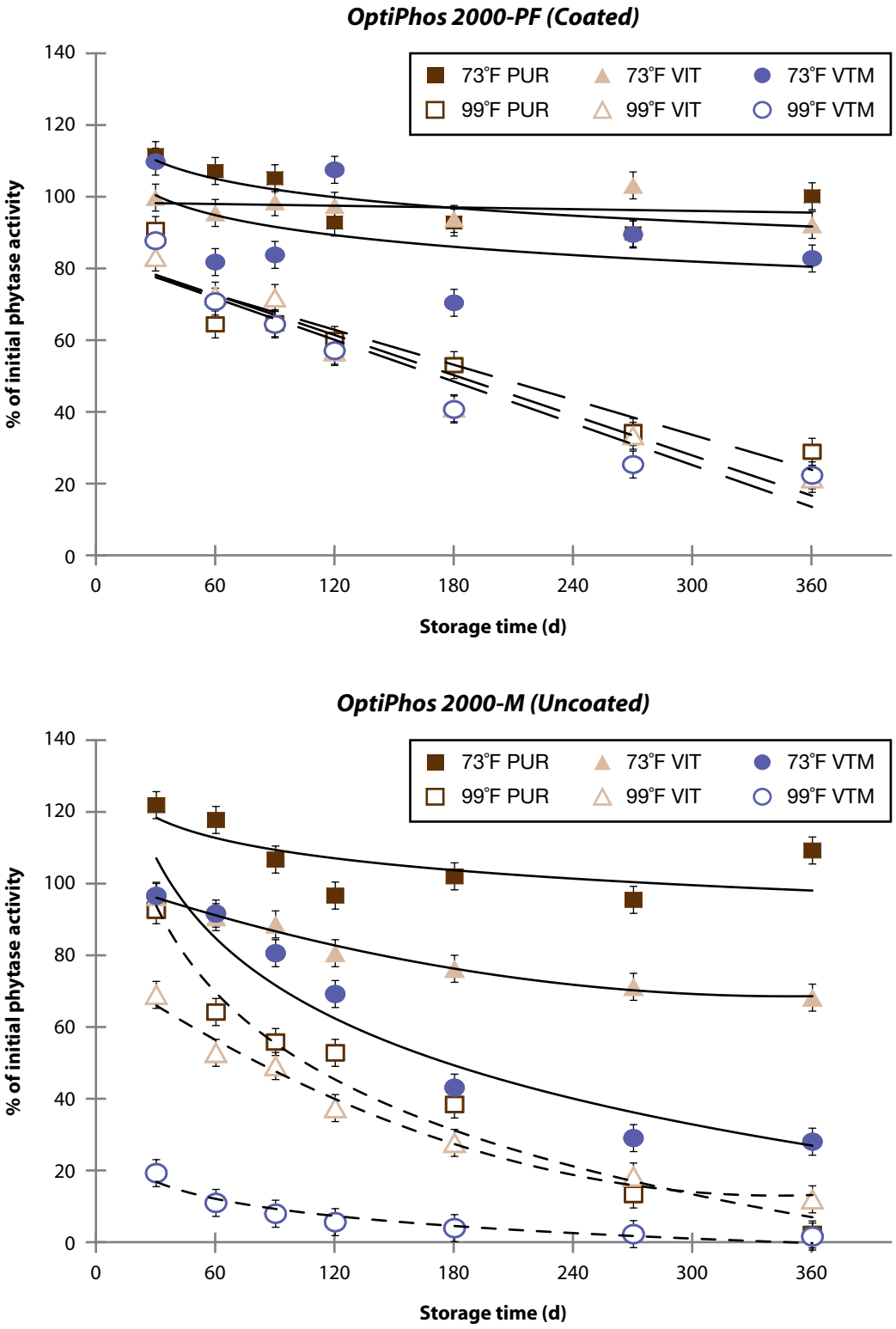

Figure 4. Residual phytase activity (% of initial) for OptiPhos 2000-PF (coated) and OptiPhos 2000-M (uncoated) as affected by form of storage (as pure product [PUR], in a vitamin premix [VIT], or in a vitamin-trace mineral premix [VTM]), storage temperature (room temperature [71°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 360 d). Each data point (least square mean ± 3.75) is the mean of 3 observations.

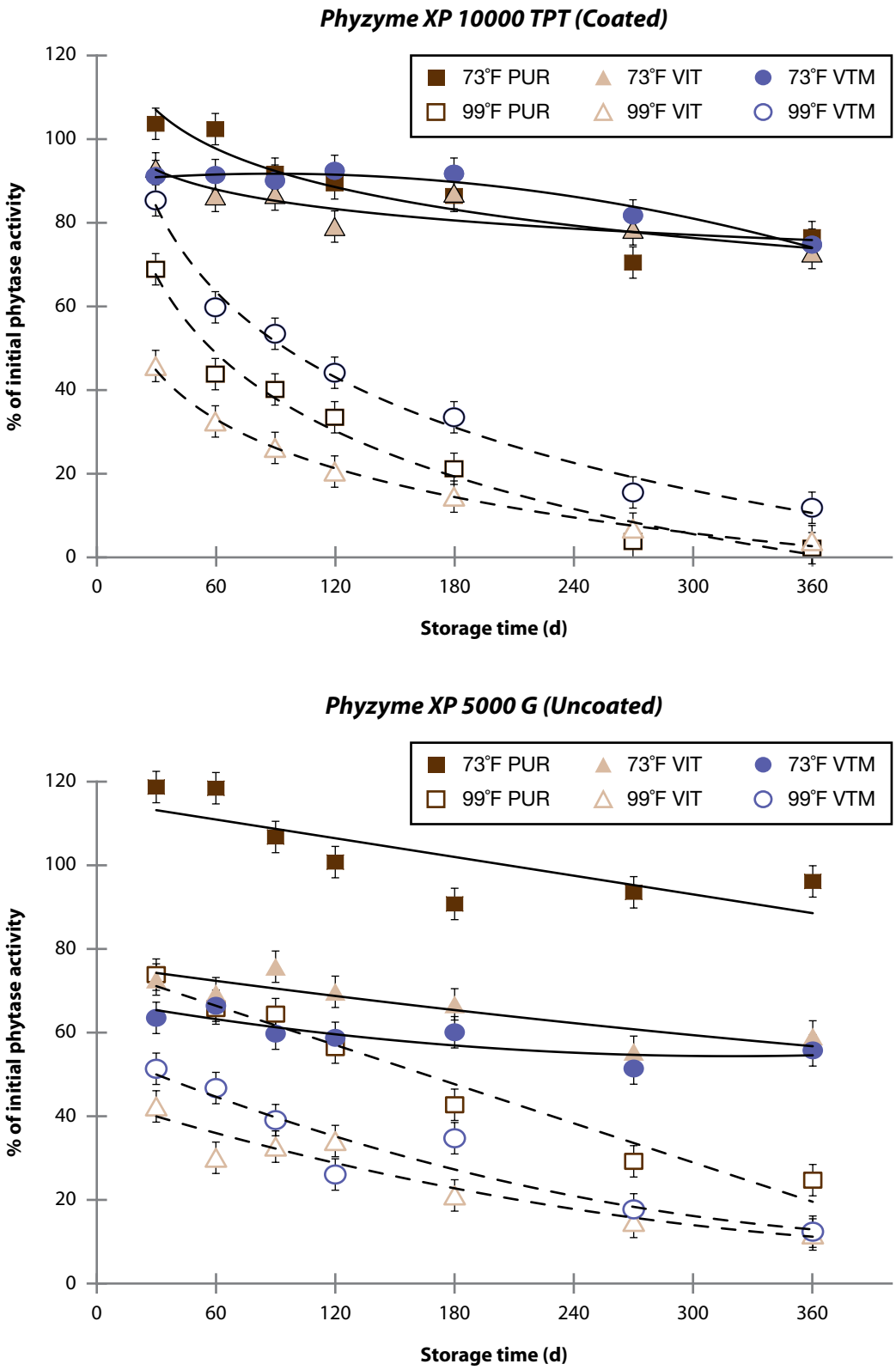

Figure 5. Residual phytase activity (% of initial) for Phyzyme 10000 TPT (coated) and Phyzyme 5000G (uncoated) as affected by form of storage (as pure product [PUR], in a vitamin premix [VIT], or in a vitamin-trace mineral premix [VTM]), storage temperature (room temperature [73°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 360 d). Each data point (least square mean ± 3.75) is the mean of 3 observations.

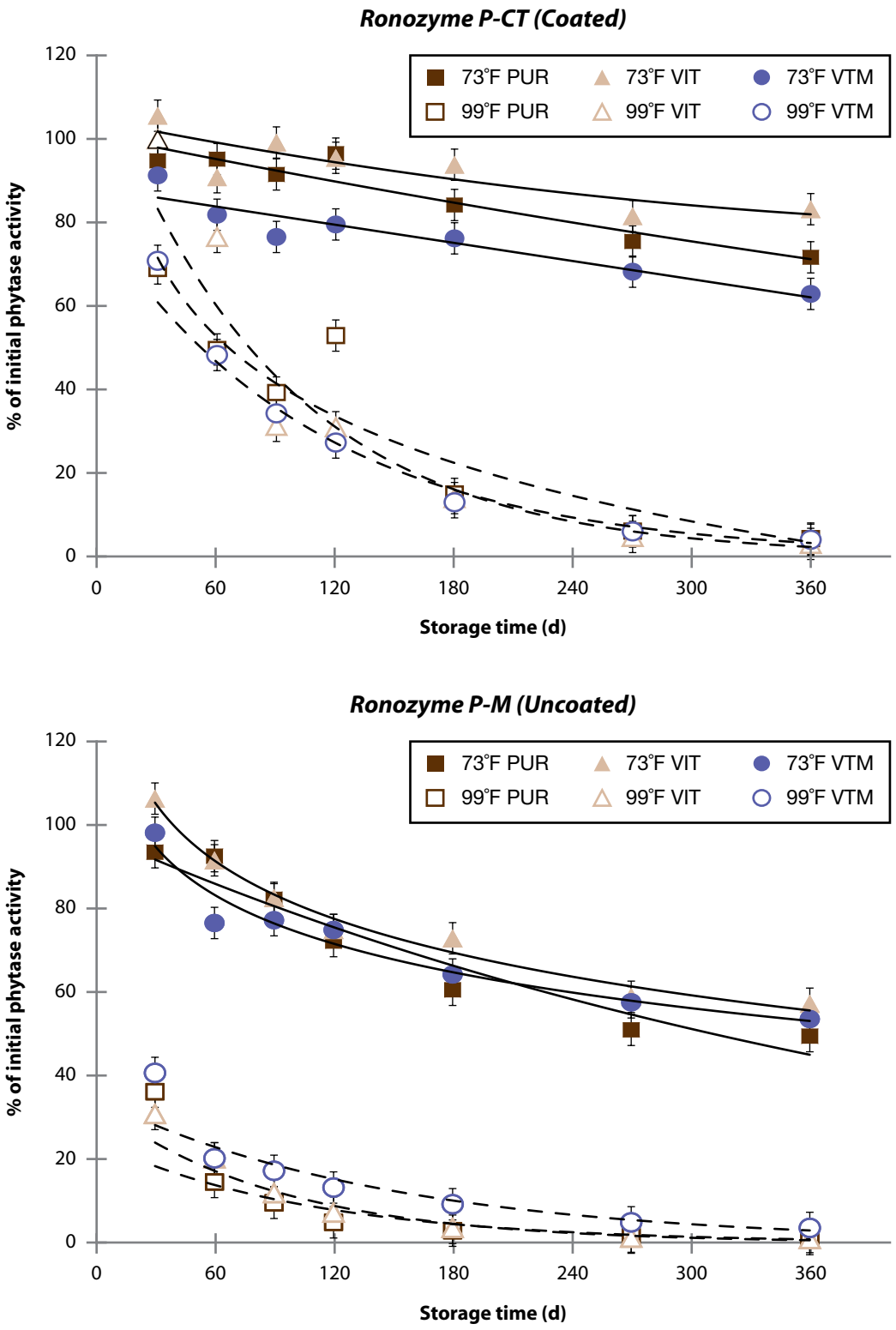

Figure 6. Residual phytase activity (% of initial) for Ronozyme CT (coated) and Ronozyme M (uncoated) as affected by form of storage (as pure product [PUR], in a vitamin premix [VIT], or in a vitamin-trace mineral premix [VTM]), storage temperature (room temperature [73°F], and in a controlled environment chamber [99°F and 75% humidity]) and time (30 to 360 d). Each data point (least square mean ± 3.75) is the mean of 3 observations.